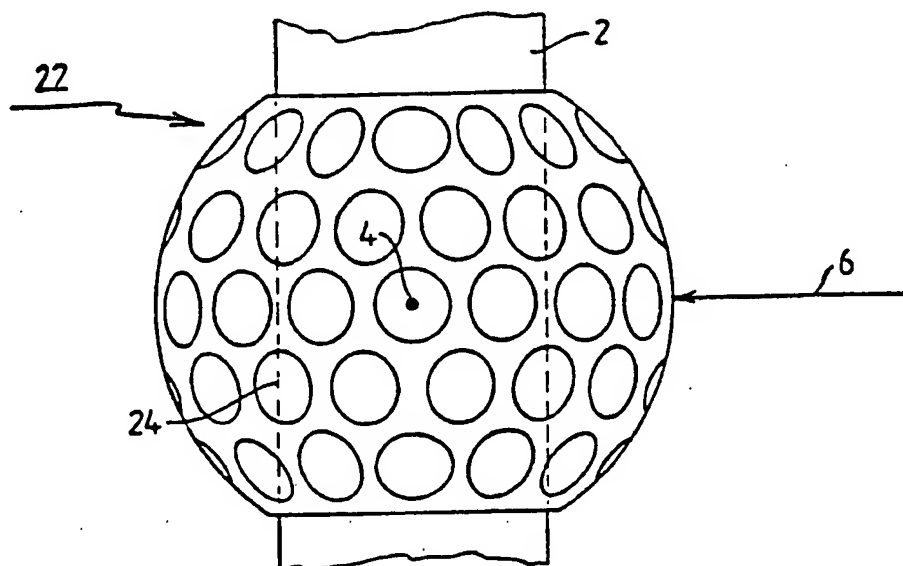




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<p>(21) International Application Number: PCT/US85/00314</p> <p>(22) International Filing Date: 26 February 1985 (26.02.85)</p> <p>(31) Priority Application Number: 3841/84</p> <p>(32) Priority Date: 29 February 1984 (29.02.84)</p> <p>(33) Priority Country: AU</p> <p>(71) Applicant: RESEARCH CORPORATION [US/US]; Suite 853, 25 Broadway, New York, NY 10004 (US).</p> <p>(72) Inventor: BOEHMER, Ralph-Michael ; Flat 4, 9 Robe Street, St. Kilda, VIC 3182 (AU).</p> <p>(74) Agent: SCOTT, Anthony, C.; Scully, Scott, Murphy & Presser, 200 Garden City Plaza, Garden City, NY 11530 (US).</p>		<p>(81) Designated States: DE (European patent), FR (Euro- pean patent), GB (European patent), JP.</p> <p>Published <i>With international search report.</i></p>

(54) Title: FLOW CYTOMETERS



(57) Abstract

A flow cytometer and a method for determining properties of single cells or other particles (40) including passing stream of particles through a zone of analysis where a light source directs a beam of light (6) to perpendicularly intersect the stream of particles so that only a single cell (4) is exposed to the light beam (6). An array of optical fibers adjacent the zone of analysis collects the light refracted by the cells as each cell (4) passes through the zone of analysis. Each fibre is connected to a photomultiplier for converting the light to electrical signals which are analyzed by an electronic analysis unit to determine the particle properties. The angle at which the light is collected by said optical fibre is adjustable to permit more light to be collected to yield more information about the particle.

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FLOW CYTOMETERSBACKGROUND OF THE INVENTION

The present invention relates to flow cytometers.

Flow cytometers (FCM) are instruments by which properties of single cells or other particles in suspension can be determined. Conventionally, an FCM consists of the following basic components:

i. A liquid flow system by which cells in suspension, which may be loaded with fluorescent dye, are transported in a vertical particle stream and passed singly, one after another, across a zone of analysis where they are exposed to an intense light beam. This zone may be located in open air or in a glass flow chamber;

ii. A light source and focussing system which directs a light beam (for example a laser beam) sharply focussed into the zone of analysis within the particle stream such that only a single cell will be exposed to the beam;

iii. An optical detection system, by which the scattered or fluorescent light pulses emitted by each cell at the moment when the cell passes across the beam, is collected, selected according to wavelength and converted into electronic pulses;

iv. An electronic analysis unit by which these pulses are processed and analyzed for the desired information about the cell characteristics which can be obtained from the light pulses.

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1 A conventional optical detection system is
shown schematically in Figure 1, which is a horizontal
section through a flow chamber of an FCM.

5 In Figure 1, the flow chamber through which
the particle stream passes is shown at 2, the section
being taken at the point at which the incident light
beam intersects the stream. The cell instantaneously
exposed to the beam is shown at 4 and the incident light
beam is shown at 6. The light pulses which are emitted
10 from the cell 4 are collected perpendicularly to the
incident beam 6 within a solid angle () by a lens 8,
then passed through a first beam splitter 10a. The
light deflected by the first beam splitter 10a is passed
through a color filter 12 onto a first photomultiplier
15 PM_1 for transformation into electronic signals. The
light transmitted through the first beam splitter 10a
meets a second beam splitter 10b. The light respective-
ly deflected and transmitted by the second beam splitter
passes through further color filters 14, 16 to further
20 photomultipliers, PM_2 and PM_3 . Thus the light pulses
are analyzed in three different parts of the wavelength
spectrum.

This conventional detection system is disadvan-
tageous in that each part of this system needs to be
25 adjusted for correct location in three dimensions, and
even with very experienced operators, initial adjust-
ments and readjustments during measurement may involve
several hours work. With systems effecting more than
three color analysis, the use of a highly skilled
30 operator is required for operation.

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1 Further, with this conventional system, all
analysis is restricted to the two dimensional plane in
which the optical system is mounted. An analysis which
could be carried on without such restriction would yield
5 more information concerning the light scatter character-
istics of cells, and a higher proportion of the omni-
directional, but normally weak, fluorescent light could
be collected.

SUMMARY OF THE INVENTION

10 According to the present invention, there is
provided an optical detection system in a flow
cytometer, comprising an array of optical fibres which
are located directly adjacent to the zone where the
light from the cell is emitted, whereby the fibres act
15 to collect emitted light.

At most, the ends of the fibres will be within
a few millimeters from the cell.

BRIEF DESCRIPTION OF THE DRAWINGS

20 The invention will now be further described,
by way of example only, with reference to the accompany-
ing drawings, in which:

Figure 1 is a schematic of a conventional
prior art optical detection system;

25 Figure 2 is a schematic horizontal cross-
-section through a flow chamber of a flow cytometer to
illustrate the basic principles of the present inven-
tion;

Figure 3 is a similar horizontal section of a
first practical embodiment of the invention;

30 Figure 4 is a side view of the embodiment of
Figure 3;

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1 Figure 5 is a horizontal section of a second
practical embodiment of the invention; and

Figure 6 is a side view of the embodiment of
Figure 5.

5 DESCRIPTION OF THE INVENTION

In accordance with the invention it has been
determined that optical fibres can be used to collect
directly the fluorescent or scattered light from the
cell. A very simple mounting system for the fibres can
10 be used which does not require a high accuracy in
setting up. More particularly, the optical fibres may
be held by the hand or fixed with a putty-like substance
about 1 mm from the flow chamber and with this form of
mounting the readings of scatter and fluorescence
15 signals obtained have been found to have the same order
of accuracy as achieved by a conventional optical system
when set up in its optimum manner.

Figure 2 shows, schematically, a horizontal
section through a transparent vertical flow chamber 2
20 through which the particle stream passes centrally, the
section being taken at the point at which the light beam
intersects the stream. The excited cell is shown at 4,
and the incident light beam is shown at 6. An optical
fibre which directly collects the emitted light is shown
25 at 20. In the configuration shown in Figure 2, the
optical fibre 20 collects light emitted from the cell 4
within a solid angle α along an axis inclined at an
angle β to the incident beam 6. As will be apparent,
simply by moving the fibre 20 toward or away from the
30 chamber 2, the measured solid angle α can be changed; a

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1 similar effect can be obtained by altering the size of
the light-acceptance aperture by means of an aperture
mask at the end of the fibre. The fibre can also be
moved in order to change the angle β relative to the
5 incident beam 6. The choice of angles α and β is not
given in conventional detection systems where both
angles are fixed.

In one practical embodiment as shown in
Figures 3 and 4, a part-spherical shell 22 is mounted
10 around part of the flow chamber 2, the center of the
sphere being coincident with the instantaneously excited
cell 4 in the chamber 2. Thus, the center of the shell
22 is coincident with the point of intersection of the
incident light beam 6 with the particle stream. The
15 beam 6 passes through an appropriate opening 23 in the
shell 22. Holes 24 are formed through the wall of the
shell 22, the axis of each hole 24 lying on a
different radial axis of the shell 22 so that each hole
24 faces toward the excited cell 4. A group of optical
20 fibres is provided (not shown), the fibres leading to
one or more photomultipliers. The ends of the fibres
can be removably plugged into any one of the holes 24 in
the shell 22 to enable readings to be taken at selected
points around the cell 4, in other words at different
25 angles of β with the possible variation of this angle
not only being in the plane of Figure 2 but also in
planes inclined to that of Figure 2. A compromise has
to be made between the desire for high angular resolu-
tion by small solid angles and the need to collect
30 sufficient amounts of light. Therefore, in practice,
the solid angle α of light collection for each photo-

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1 multiplier also needs to be variable. Possible methods
of varying the solid angle include the following:

5 a. Different sizes of holes 24 for fitting
different diameter fibres. This would require a pre-
determination of angles of interest for the scatter
light analysis, where the angle of resolution is im-
portant, the remaining angles being free for larger size
fibres collecting the omnidirectional fluorescent light.

10 b. Fibre fittings for allowing variation of
depth of fibre plugging, thus varying the angle of light
acceptance by the distance of the light acceptance
aperture from cell 4.

15 c. Photomultipliers for allowing collective
entry of many fibres, so that for weak fluorescent
light, the light from different directions may be
collected by several fibres and directed into one
photomultiplier.

20 Since fibres are relatively inexpensive, the
fibres may be fixedly mounted in the shell 22. In this
embodiment each hole 24 is non-removably plugged with a
fibre, with the selection of light analysis angles being
obtained by plugging the other ends of the relevant
fibres into selected photomultipliers. This would
facilitate the precision-setting of all fibres on the
shell 22 and thus reduce alignment problems.

25 The shell 22 may be supported by a mounting
system which allows adjustment of the position of the
shell 22 in all directions relative to the flow chamber.

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1 Alternatively, the shell 22 may be mounted by a precision lock in a fixed position relative to the flow chamber, to thereby avoid the necessity of having to align the system subsequent to manufacture.

5 It is to be noted that the flow chamber 2 is not of conventional rectangular cross-section, but in the embodiment shown is of circular cross-section, the chamber being of cylindrical form. Alternatively, the chamber may be of spherical form, with the entry and exit areas of the incident light beam being flattened.
10 In this case all non-perpendicular transitions of light through the interface between glass and air would be avoided. However, the use of a flow chamber is not essential, and the system shown in Figures 3 and 4 can be used in an FCM in which the particle stream moves
15 through open air.

In another practical embodiment, as shown in Figures 5 and 6, the chamber 2 extends through a parabolic reflective shell 30 with the instantaneously excited cell 4 being at the focus of the parabola. This
20 parabolic shell 30 is closed by a circular plate 32 the center of which is apertured for passage of the incident light beam 6 onto the cell 4 at the focus of the shell 30. The shell itself is provided with an aperture 33 in alignment with the central aperture in the plate to
25 permit exit of the light beam 6. Holes 34 are formed through the plate 32 in a number of concentric rows. With each hole 34 being directed perpendicularly to the plane of the plate 32, i.e. parallel to the light beam
30 6.

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1 Due to the parabolic form of the reflective
shell 30, the scattered and fluorescent light will be
reflected parallel to the axis of the parabola, that is
at right angles to the plate 32 and parallel to the
incident beam 6. Optical fibres leading to photo-
5 multipliers can be plugged into selected ones of the
holes 34. As will be apparent each concentric row of
holes will be associated with light scattered at the
periphery of discrete cone angles, and fibres plugged
into the respective rings will collect light at dif-
10 ferent points around the relevant cone angle. In
effect, the use of the parabolic shell enables the
collection of the light emitted all around a certain
cone angle and this can be detected with high angular
resolution despite an overall large area of light
15 collection due to rings of fibres.

 Instead of using a reflective paraboloid
separate from the flow chamber, it might be advantageous
to shape the whole flow chamber accordingly and provide
the chamber with a reflective coating. This would avoid
20 non-perpendicular transition of light through interfaces
of media with different refractive indices (glass-air)
and thus avoid reflection and beam-shift problems.

 The use of a fixed or movable mounting system
for the shell itself and of removable or non-removable
25 fibres as discussed in connection with the previous
embodiment, applies to this embodiment also.

 In the two practical embodiments described,
color discrimination filters can be associated with the
fibres, the filters preferably being positioned at the
30 point where the fibres enter the housing of the photo-
multipliers.

35

1 If light polarization studies are to be
performed, polarizing filters must be applied before the
light enters the fibres because of the depolarizing
effect of fibre light conductors.

5 The use of optical fibres to directly collect
the emitted light provides enhanced flexibility of
measurement in relation to that of a conventional
optical system, and permits easier setting up of experi-
ments. More specifically, the main advantages of the
described systems are:

- 10 i. Reduction of optical alignment problems;
 ii. Reduced need for highly skilled personnel
for operating the system;
 iii. Reduced cost of flow cytometers;
15 iv. Increased versatility for sophisticated
non-routine investigations on cell discrimination.

20 While illustrative embodiments of the subject
invention have been described and illustrated, it is
obvious that various changes and modifications can be
made therein without departing from the spirit of the
present invention which should be limited only by the
scope of the appended claims.

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1 WHAT IS CLAIMED IS:

1. A flow cytometer comprising:
means for transporting a stream of particles
having cells in suspension such that each cell passes
5 singly through an analysis zone;
a light source for directing a light beam that
perpendicularly intersects said particle stream at said
analysis zone such that only a single cell will be
exposed to said light beam;
10 an optical detection means including at least
one optical fibre located adjacent said analysis zone
for collecting the light emitted by each cell as each
cell passes through said analysis zone; and
electronic means connected to said optical
15 fibres for converting the light collected by said
optical fibres into electrical impulses and analyzing
said impulses for the desired information.
2. The flow cytometer of Claim 1, wherein
said optical detection means includes an array of
20 optical fibres.
3. The flow cytometer of Claim 1 or 2,
wherein each of said optical fibres collects the light
emitted by the cells within a collection angle defined
by the cell and the outer perimeter of the optical fibre
and at an incident angle defined by the light beam and
25 the longitudinal axis of the optical fibre.
4. The flow cytometer of Claim 3, wherein
said collection angle is adjustable by changing the
distance between said cell and said optical fibres.
- 30 5. The flow cytometer of Claim 3, wherein
each of said optical fibres include an aperture mask for
adjusting the collection angle.

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1 6. The flow cytometer of Claim 1, 2 or 3,
wherein said optical detection means includes a shell
mounted around the analysis zone, said shell having an
opening for allowing said light beam to pass through
5 said shell into said analysis zone, said shell further
including a plurality of apertures for receiving at
least one optical fibre.

 7. The flow cytometer of Claim 6, wherein
said shell is substantially spherical in configuration
10 with the center of said sphere being coincident with the
cell in the analysis zone and wherein the axis of each
aperture lying on a different radial axis of said shell
so that each aperture faces toward the cell in the
analysis zone.

15 8. The flow cytometer of Claim 6, wherein
said shell is partially parabolic in configuration
including a partial parabolic reflective surface ending
at a flat circular plate positioned perpendicular to
said beam of light wherein the focus of said parabola
20 being coincident with the cell in the analysis zone,
said flat circular plate having said plurality of
apertures and said opening for the light beam.

 9. The flow cytometer of Claim 8 wherein said
apertures are formed in concentric rows with said
opening in the center thereof, each of said apertures
25 being directed perpendicular to the plane of said
circular plate.

 10. The flow cytometer of Claims 6, 7, 8, or
9, wherein said optical fibres are removably mounted
30 within said apertures thereby permitting light to be
collected at different incident angles.

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11. The flow cytometer of Claims 6, 7, 8, or
1 9, wherein said apertures are of several different sizes
for receiving optical fibres of different diameters
thereby varying the collection angle.

12. The flow cytometer of Claims 6, 7, 8, or
5 9, wherein said optical fibres are adjustably mounted
within said apertures allowing variation in the depth
said optical fibres are received in each aperture
thereby varying the collection angle by varying the
10 distance between said cell and said optical fibre.

13. The flow cytometer of Claims 6, 7, 8, or
9, wherein a plurality of said optical fibres are
connected to a photomultiplier for allowing light from
different incident and collection angles to be analyzed
15 together.

14. The flow cytometer of Claims 6, 7, 8, or
9, wherein a plurality optical fibres are rigidly
secured in the apertures of said shell and a variation
in said incident and collection angles being obtained by
20 connecting the optical fibres to a plurality of selected
photomultipliers.

15. The flow cytometer of Claims 6, 7, 8, or
9, wherein said shell is adjustably mounted to allow
adjustment of the position of said shell relative the
analysis zone.
25

16. The flow cytometer of Claims 6, 7, 8, or
9, wherein said shell is secured in a fixed position
relative to said analysis zone.

17. The flow cytometer of Claim 1, wherein
30 said transporting means includes a flow chamber through
which said particle stream passes, said analysis zone
being within said flow chamber.

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1 18. The flow cytometer of Claims 6, 7, or 8,
wherein said transporting means includes a flow chamber
through which said particle stream passes, said analysis
zone being within said flow chamber, said flow chamber
being within said shell.

5 19. The flow cytometer of Claim 1, wherein
said transporting means includes a flow chamber through
which said particle stream passes, said analysis zone
being within said flow chamber, said flow chamber having
10 a partially parabolic surface having a reflective
coating thereon, said partial parabolic surface ending
at a flat circular plate having an opening for allowing
said light beam to pass through.

15 20. The flow cytometer of Claim 19, wherein
said flat circular plate of said flow chamber includes a
plurality of apertures for receiving said optical fibre.

 21. The flow cytometer of claims 6, 7 or 8
wherein said electronic means includes at least one
photomultiplier.

20 22. The flow cytometer of Claim 21 further
including a color discriminating filter connected to
each of said fibres.

25 23. The flow cytometer of Claim 22 wherein
said color discriminating filters are positioned at the
point where said fibres enter said photomultipliers.

 24. A method for determining the properties
of particles comprising:

 passing a stream of particles through a zone
of analysis;

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1 directing a light beam perpendicularly intersecting same stream of particles, said light being refracted by said particles as each particle passes through the zone of analysis;

5 collecting the refracted light with at least one optical fibre;

electronically analyzing said collected refracted light to determine the desired information.

25. The method of Claim 24, wherein said stream of particles includes cells in suspension.

10 26. The method of Claim 25, wherein said cells are singly passed through said zone of analysis and said light beam is directed such that only a single cell is exposed to said beam.

15 27. The method of Claim 24, further including the step of adjusting the distance between said optical fibre and said zone of analysis.

28. The method of Claim 24, wherein said refracted light is collected by an array of optical fibres.

20 29. The method of Claim 28, wherein said array of optical fibres are removably and adjustably mounted to a shell surrounding said zone of analysis.

25 30. The method of Claim 28, wherein said array of optical fibres are fixedly mounted to a shell surrounding said zone of analysis.

31. The method of Claim 24, wherein said zone of analysis is within a flow chamber.

30 32. The method of Claim 28 or 29, further including reflecting the light refracted by said particle off the inner surface of said shell and wherein said array of optical fibres collects said reflected light.

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- 1 33. The method of Claim 24, further including the step of connecting said optical fibre to a photo-multiplier for connecting said collected light into a series of electrical pulses.

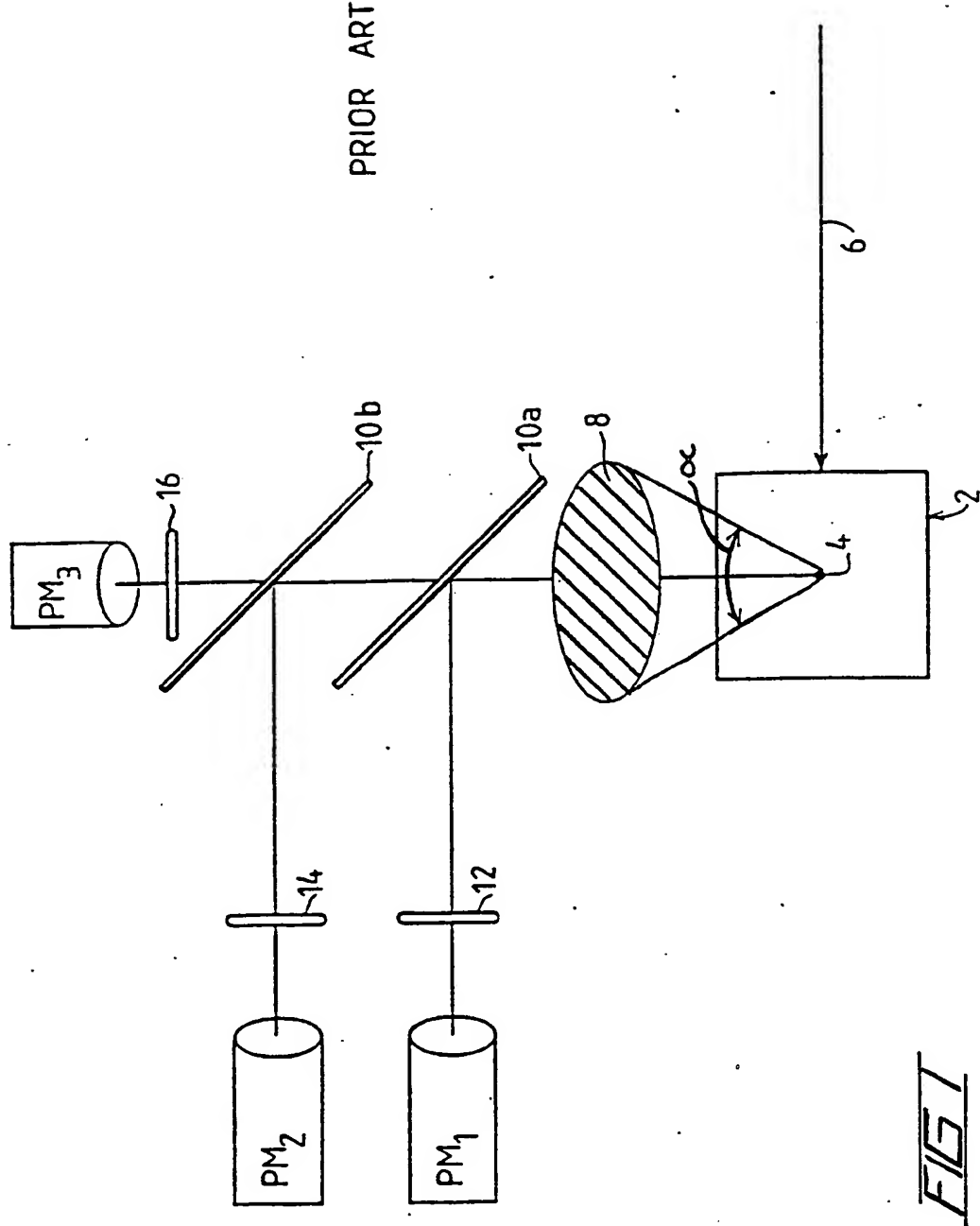
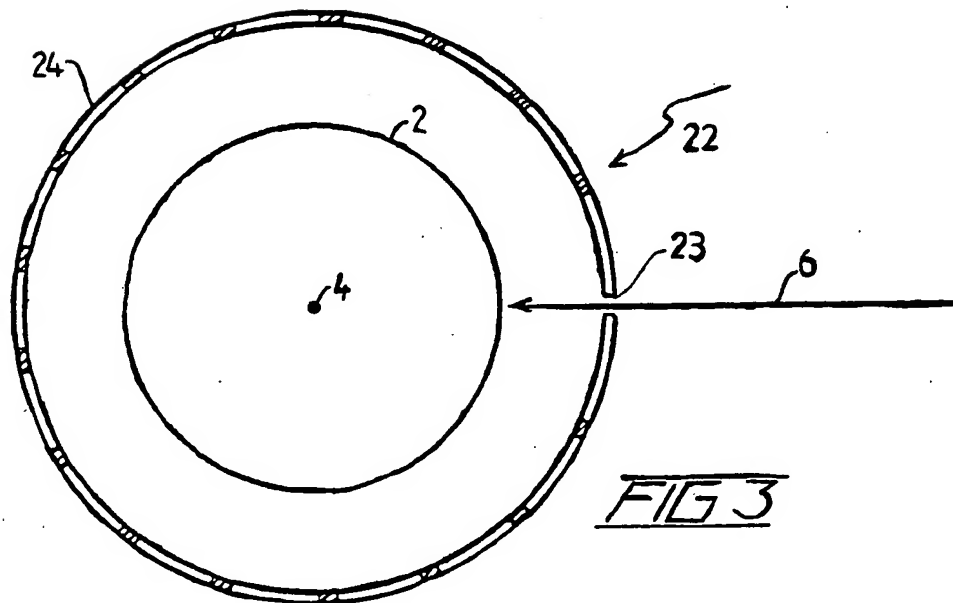
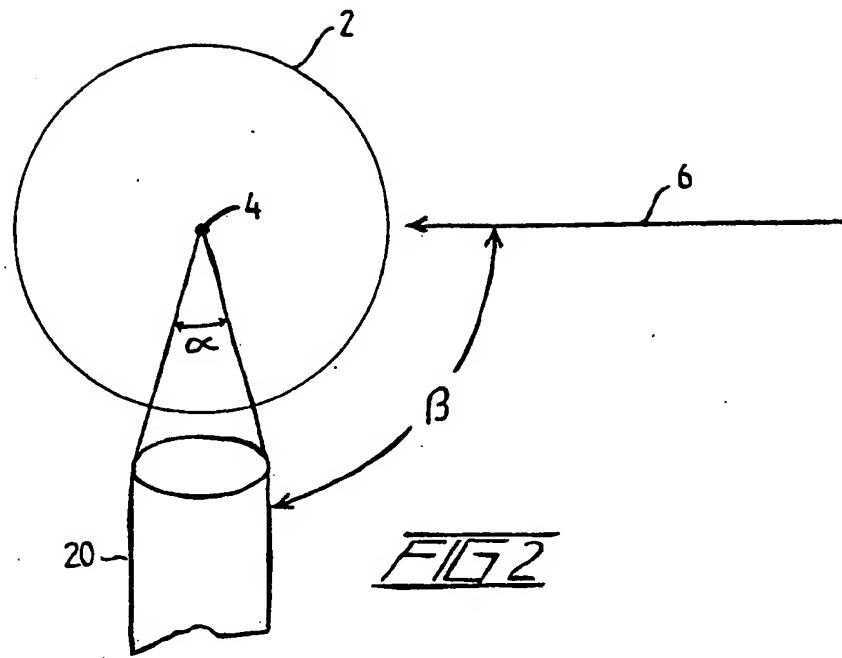


FIG 1



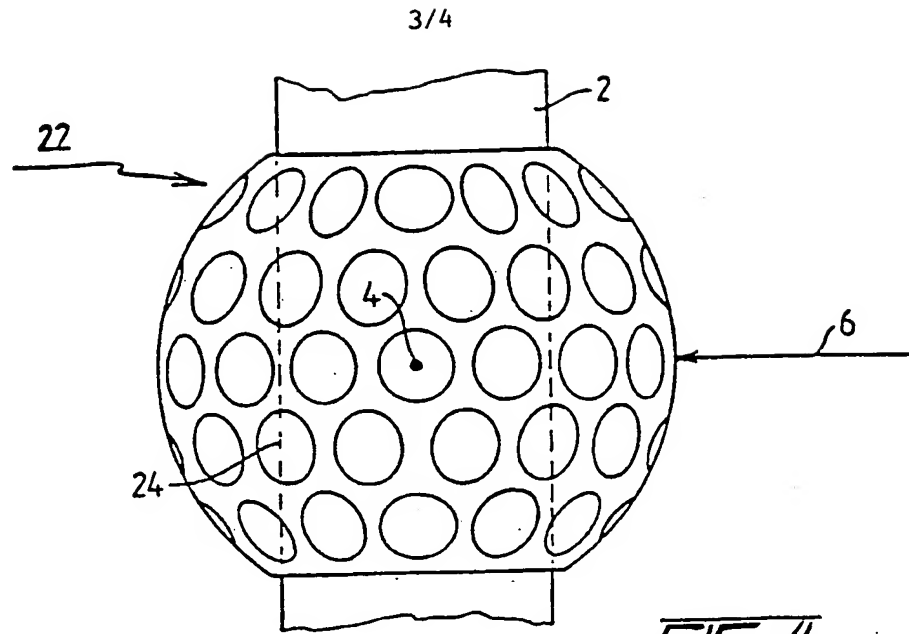


FIG 4

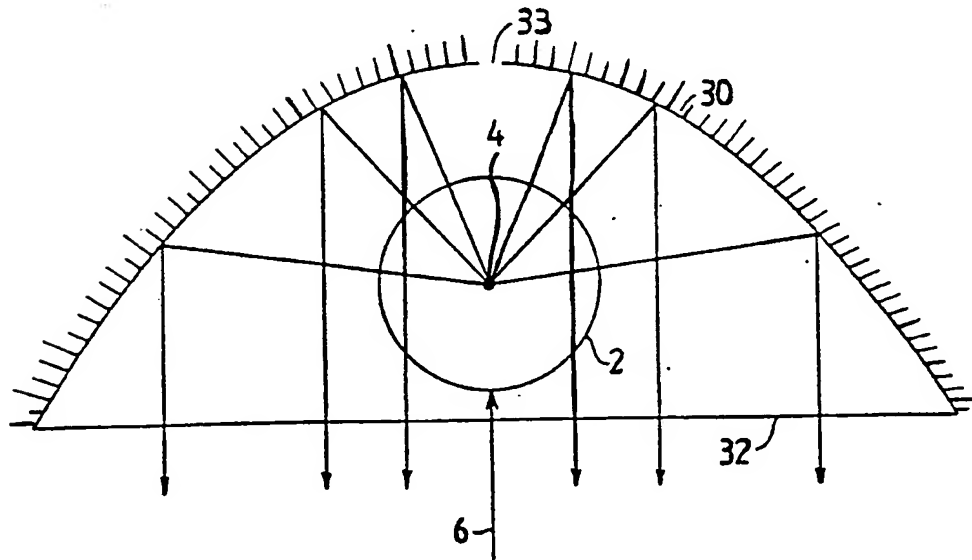
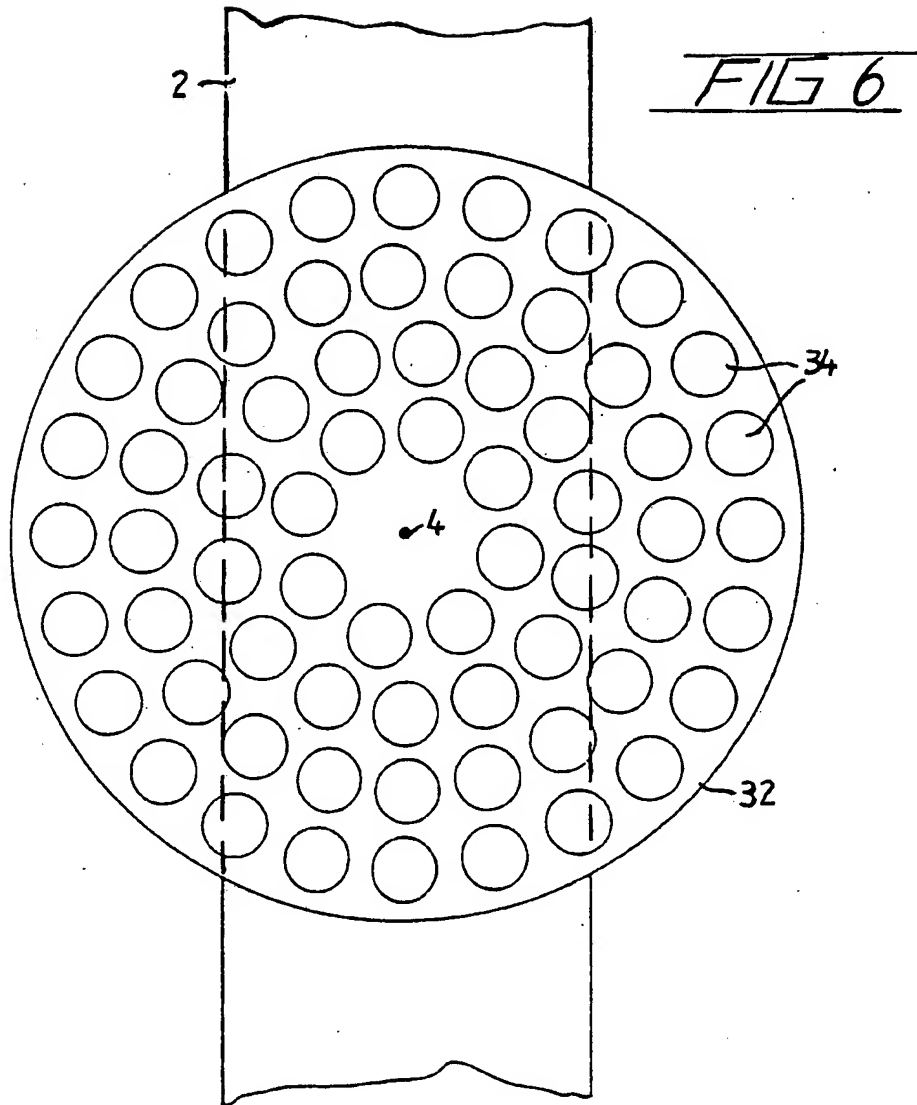


FIG 5

4/4.



INTERNATIONAL SEARCH REPORT

PCT/US85/00314

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ³ According to International Patent Classification (IPC) or to both National Classification and IPC <div style="text-align: center; font-weight: bold;"> INT. CL. 4 G01N 21/00 U.S. CL. 356/73, 340 </div>																										
II. FIELDS SEARCHED <div style="text-align: center; font-weight: bold;">Minimum Documentation Searched ⁴</div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%;">Classification System</th> <th style="width: 80%;">Classification Symbols</th> </tr> <tr> <td style="text-align: center; vertical-align: top;">US</td> <td style="vertical-align: top;">356/39, 72, 73, 336, 37, 338, 339, 340, 341, 343 250/227</td> </tr> </table> <div style="text-align: center; font-weight: bold; margin-top: 5px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵</div>			Classification System	Classification Symbols	US	356/39, 72, 73, 336, 37, 338, 339, 340, 341, 343 250/227																				
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US	356/39, 72, 73, 336, 37, 338, 339, 340, 341, 343 250/227																									
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴ <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%;">Category [*]</th> <th style="width: 60%;">Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷</th> <th style="width: 30%;">Relevant to Claim No. ¹⁸</th> </tr> <tr> <td style="text-align: center;">A</td> <td>US, A, 3,781,112, Published 25 December 1973, Groner et al.</td> <td style="text-align: center;">1-33</td> </tr> <tr> <td style="text-align: center;">A</td> <td>US, A, 4,101,276, Published 18 July 1978 Anderson</td> <td style="text-align: center;">1-33</td> </tr> <tr> <td style="text-align: center;">Y</td> <td>US, A, 4,175,865, Published 27 November 1979 Horvath et al.</td> <td style="text-align: center;">1-33</td> </tr> <tr> <td style="text-align: center;">Y</td> <td>US, A, 4,200,802, Published 29 April 1980 Salteman et al.</td> <td style="text-align: center;">1-33</td> </tr> <tr> <td style="text-align: center;">Y</td> <td>US, A, 4,201,471, Published 6 May 1980 Pitt et al.</td> <td style="text-align: center;">1-33</td> </tr> <tr> <td style="text-align: center;">Y</td> <td>US, A, 4,250,394, Published 10 February 1981 O'Connor</td> <td style="text-align: center;">1-33</td> </tr> <tr> <td style="text-align: center;">A</td> <td>US, A, 4,348,107, Published 7 September 1982 Leif</td> <td style="text-align: center;">1-33</td> </tr> </table>			Category [*]	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸	A	US, A, 3,781,112, Published 25 December 1973, Groner et al.	1-33	A	US, A, 4,101,276, Published 18 July 1978 Anderson	1-33	Y	US, A, 4,175,865, Published 27 November 1979 Horvath et al.	1-33	Y	US, A, 4,200,802, Published 29 April 1980 Salteman et al.	1-33	Y	US, A, 4,201,471, Published 6 May 1980 Pitt et al.	1-33	Y	US, A, 4,250,394, Published 10 February 1981 O'Connor	1-33	A	US, A, 4,348,107, Published 7 September 1982 Leif	1-33
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A	US, A, 4,348,107, Published 7 September 1982 Leif	1-33																								
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents: ¹⁶</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>																										
IV. CERTIFICATION <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; vertical-align: top;"> Date of the Actual Completion of the International Search ³ <div style="text-align: center; font-weight: bold;">7 May 1985</div> International Searching Authority ¹ <div style="text-align: center; font-weight: bold;">ISA/US</div> </td> <td style="width: 50%; vertical-align: top;"> Date of Mailing of this International Search Report ³ <div style="text-align: center; font-weight: bold; font-size: 1.2em;">13 MAY 1985</div> Signature of Authorized Officer ¹⁹ </td> </tr> </table>			Date of the Actual Completion of the International Search ³ <div style="text-align: center; font-weight: bold;">7 May 1985</div> International Searching Authority ¹ <div style="text-align: center; font-weight: bold;">ISA/US</div>	Date of Mailing of this International Search Report ³ <div style="text-align: center; font-weight: bold; font-size: 1.2em;">13 MAY 1985</div> Signature of Authorized Officer ¹⁹ 																						
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